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NUTRITION REVIEWS

May 1946

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NUTRITION REVIEWS

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NUTRITION IN THE PUBLIC HEALTH PROGRAMS

Proper nutrition plays a vital role in building and maintaining healthy and vigorous nations. It is significant that the Food and Agriculture Organization is the first permanent international body to develop from the wartime cooperation of the United Nations, being an outgrowth of the first United Nations Conference on Food and Agriculture at Hot Springs in 1943. It was emphasized then that we cannot maintain freedom from fear unless we conquer hunger and bring to every individual the ordinary needs of life. It was recognized also that each country should be responsible for providing its people with the food needed for life and health, but this goal cannot be achieved without international cooperation.

During the war the importance of nutrition in the prevention of disease was clearly demonstrated. The amazingly good national wartime health in Great Britain was attributed to the British nutrition program. Though monotonous and restricted, the British diet supplied necessary nutrients to more people than before the war, and emphasis was placed upon the needs of infants, young children, pregnant women, and nursing mothers. Sir William Jameson, chief medical officer of the British Ministry of Health, gives credit to food planning for keeping Britain's death rate down, for keeping the infant mortality rate the lowest on record, and for the absence of serious epidemic disease despite bombings, mass evacuations, and the strain of war.

In this country, praise is due the Nutrition Programs Branch of the War Food Administration for coordination of nutrition education activities in keeping our people strong and well nourished during the war. Statewide nutrition committees set up under this program brought together representatives of agriculture, welfare agencies, community groups, and health officers.

The prevention of dietary deficiency disease presents increased responsibility to public health departments. We are discovering every day that this is a field of preventive medicine as great as the development of sanitation or the control of communicable disease. Further, malnutrition, as we know, is an underlying factor in other health problems such as tuberculosis. Nutrition is also related to the general mortality rate, especially in children. No community is sufficiently free of malnutrition to be complacent. Nutrition surveys and dietary studies of selected population groups reveal that although malnutrition is most prevalent among low economic groups, it is also found among people who have sufficient money to be well fed.

Great progress in research has been made and this increased knowledge has been reflected in the fact that most public health officials now appreciate the importance of nutrition in preventive medicine. One of the health officer's great difficulties, however, is that of accurate diagnosis of dietary deficiency disease at an early stage. There must be improved laboratory methods as well as better interpretation of early clinical findings to help in proper and immediate diagnosis of malnutrition before it reaches the point of severe dietary deficiency disease. Another great need of the health officer is knowledge concerning the prevalence of malnutrition in the community. At the present time there is no generally agreed opinion of the amount of dietary deficiency disease which actually exists in the United States. We know that reported deaths from pellagra, beriberi, severe infantile rickets, or scurvy are not a true measure of the

public health nutrition problem. For each death reported, no doubt there are many sufferers who recover, in addition to the many mild, unrecognized cases of malnutrition. It is essential that health officers collect information to help appraise the distribution, incidence, and types of deficiency diseases. In this role the health officer requires the cooperation of agriculture, welfare, and community groups.

A program to educate communities and individuals in sound nutrition principles is necessary. This education could be accomplished through attractive literature, the press and radio, films, demonstrations, and in stimulating cooperative programs with local organizations. Health officers also should work closely with the local medical and dental professions in community nutrition problems and practical solutions. Every effort should be made to offer nutrition education to patients attending public health clinics. Health officers can offer a consultative service encouraging public eating places to serve food of good nutritional value and to conserve nutrients in food preparation.

In the development of well rounded nutrition programs, special attention must be given to certain vulnerable population groups such as pregnant and nursing mothers, schoolchildren, and industrial workers. During pregnancy and lactation, steps should be taken to provide necessary food, either free or at low cost for those unable to pay. All schoolchildren should be able to obtain a carefully planned mid-day meal, providing at least one third of the day's food needs, and in physical examinations of school and preschool children special attention should be given to evidence of nutritional deficiencies. Action to provide an adequate school meal and nutrition education to every schoolchild would be one of the greatest single forward steps in nutrition. Well rounded programs of industrial hygiene must emphasize adequate foods to keep workers healthy and strong.

We in the U. S. Public Health Service are particularly interested in assisting state and local health departments in building strong nutrition programs. Recently by appropriation the Congress made it possible for the Public Health Service to expand its activities in the field of nutrition. A nutrition section has been set up in the States Relations Division to give consultation services to the states and to develop nutrition demonstration projects for localities on invitation from a state. The purpose of these demonstration units will be to make general nutritional appraisals of the population and to develop methods and procedures particularly adaptable to health departments. Each of these nutrition teams will include a medical officer, a public health nurse, a nutritionist, and a biochemist. They will be fully equipped with mobile laboratories.

All of us in public health and other groups interested in nutrition must strengthen the fight against one of the twentieth century's most dangerous health enemies—malnutrition. We need a sound food and nutrition policy and a national nutrition organization to collaborate fully with the Food and Agriculture Organization to accrue benefits of good nutrition not only for our people but for other nations.

THOMAS PARRAN,
Surgeon General,
U. S. Public Health Service.

PRESENT KNOWLEDGE OF THE VITAMIN B-COMPLEX IN HUMAN NUTRITION (PART I)

There is not complete agreement among specialists in the field as to what substances should properly be included in a list of the recognized B-vitamins. It is probable that

all authorities would agree on the inclusion in such a list of thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, and Lactobacillus casei factor ("folic acid," "vitamin M," "vitamin Bc"). Choline and inositol are considered by most investigators to be vitamins, and a few individuals place para-aminobenzoic acid in the same category.

Of these ten compounds only three, thiamine, riboflavin, and niacin, have been accepted by the Council of Pharmacy and Chemistry of the American Medical Association as having recognized clinical value ("New and Nonofficial Remedies," *pages 594–598, American Medical Association, Chicago (1945)*). Pyridoxine is accepted "to assure the availability of a preparation of satisfactory composition for investigational use" but definite claims as to its clinical value are not as yet permitted.

The value of thiamine in the prevention and correction of beriberi is well established. It appears definite that in this disease the signs and symptoms which are referable to the heart as well as the polyneuritis are ascribable to a deficiency of thiamine. The administration of this vitamin to patients with "wet" beriberi, characterized by signs of heart failure, is followed by a decrease in cardiac size and by the dramatic relief of certain symptoms. Experimentally induced thiamine deficiency leads to electrocardiographic changes both in experimental animals and in man. In experimental animals, the deficiency has been observed to progress to myocardial necrosis (*Nutrition Reviews* **1,** *314 (1943)*) and cardiac failure (*Ibid.* **1,** *139 (1943)*). The belief that the polyneuritis of "dry" beriberi is a manifestation of thiamine deficiency has been given experimental support by the work of R. D. Williams and associates at the Mayo Clinic (*Ibid.* **1,** *252 (1943)*). The appearance of polyneuritis in human subjects was shown by these investigators to depend on the severity of the thiamine restriction. Too mild a restriction (0.22 mg. of thiamine per 1000 calories) resulted in impairment of mental and physical health with no detectable involvement of peripheral nerves and too severe a restriction (0.075 mg. per 1000 calories) led to severe inanition and prostration which necessitated discontinuation of the experiment. An intermediate level however (0.1 mg. per 1000 calories) resulted in peripheral neuritis. Results of similar import have been obtained with various experimental animals (*Ibid.* **3,** *21 (1945)*). In addition to the polyneuritis of beriberi, thiamine is of proved value in the treatment of alcoholic neuritis (*Ibid.* **1,** *148 (1943)*) and the neuritis of pregnancy and of pellagra. Experimental thiamine deficiency in man has led invariably to anorexia and to certain mental or personality changes. The subjects became depressed, irritable, quarrelsome, uncooperative, and fearful.

The question of the quantitative human requirement for thiamine remains controversial. The figure accepted by the U. S. Food and Drug Administration is 1 mg. per person per day. A. Keys, E. W. McHenry, L. E. Holt, and their co-workers place the requirement at about 0.23 mg. per 1000 calories (*Nutrition Reviews* **2,** *140, 264 (1944)*; **3,** *180 (1945)*). V. A. Najjar and L. E. Holt (*Ibid.* **2,** *109 (1944)*) claim to have restricted young men to 0.1 to 0.2 mg. of thiamine per person per day for months with no signs of deficiency. This, it is stated, was followed by complete omission of thiamine from the diet for seven weeks during which time 4 of the 9 subjects remained symptom free. The authors explained these results on the basis of irregular synthesis of thiamine by intestinal bacteria. R. D. Williams, E. E. Foltz, and their associates, on the other hand (*Ibid.* **1,** *46 (1942)*; **1,** *295 (1943)*; **2,** *264, 328 (1944)*) place the human requirement for thiamine at a higher figure. Williams and co-workers observed clinical manifestations of thiamine deficiency on a diet supplying 0.22 mg. of the vitamin per 1000 calories, while Foltz and associates reported the development of deficiency signs

in subjects receiving 0.33 to 0.38 mg. of thiamine per 1000 calories. It is impossible at the present time to reconcile these conflicting results. There is considerable reason to doubt that man derives any significant portion of his thiamine from bacterial synthesis in the intestines (*Ibid.* **3,** *147* (*1945*)). Many of the experiments cited above were short-term in nature, also, and the question of body stores cannot be entirely disregarded. For the moment, it appears necessary to reserve judgment as to the precise amount of thiamine which is essential for man.

In the foregoing we have been concerned with the requirement of "normal" individuals ingesting diets of average carbohydrate content. It is recognized that the absolute requirement for thiamine is increased in conditions involving augmented metabolism and in conditions involving faulty assimilation. It is recognized further, that the need for thiamine is determined to a very large extent by the amount of carbohydrate to be metabolized rather than by the total caloric intake. The only known function of thiamine in the body is to assist, as thiamine pyrophosphate (cocarboxylase), in the metabolism of carbohydrate (more specifically, in the metabolism of pyruvic acid). It is not surprising, therefore, that with experimental animals the replacement of carbohydrate by fat, or by protein (*Nutrition Reviews* **1,** *172* (*1943*)), in diets low in thiamine, has been found to delay the onset of deficiency signs. In even more striking experiments, rats were maintained for a year on a thiamine free diet which contained 80 per cent of protein and no carbohydrate. The rats did not grow rapidly but otherwise at the end of the year they appeared to be in excellent condition (W. J. Dann, *Federation Proceedings* **4,** *153* (*1945*)). Contrary to previous beliefs, thiamine does not appear to be needed in the metabolism of alcohol (*Nutrition Reviews* **1,** *101* (*1943*); **3,** *356* (*1945*)). It follows that alcoholic neuritis in all likelihood results from a low intake (or from faulty assimilation) of thiamine rather than from increased requirements.

The Recommended Dietary Allowances adopted by the Food and Nutrition Board of the National Research Council in May, 1941, included values for thiamine of approximately 0.6 mg. per 1000 calories (*Nutrition Reviews* **1,** *164* (*1943*)). In 1945 they were revised downward to approximately 0.5 mg. per 1000 calories (*Ibid.* **3,** *287* (*1945*)). These "allowances" should not be confused with "requirements." It will be remembered that the allowances were designed "to provide not merely the minima sufficient to protect against actual deficiency disease but a fair margin above this to insure good nutrition and protection of all body tissues."

The signs of riboflavin deficiency in man first observed by W. H. Sebrell and R. E. Butler included glossitis, cheilosis, and seborrheic dermatitis (*Nutrition Reviews* **1,** *327* (*1943*)). H. D. Kruse *et al.* later described ocular manifestations of human ariboflavinosis (*Ibid.* **1,** *194* (*1943*)). A great deal of discussion has centered about the specificity of these signs and the prevalence of riboflavin deficiency in various population groups. Our knowledge along these lines is still far from complete. There appears to be no doubt that the signs and symptoms described may be caused by riboflavin deficiency but it is admittedly difficult and probably impossible to diagnose ariboflavinosis on this basis alone. Signs such as glossitis, cheilosis, dermatitis, and corneal vascularization are not, of course, restricted to deficiency diseases (*Ibid.* **1,** *194* (*1943*); **2,** *28* (*1944*); **3,** *133*, *235* (*1945*)) and not all are restricted, among the deficiency diseases, to ariboflavinosis. Criteria have been presented which are said to be helpful in distinguishing the glossitis of riboflavin deficiency from that of pellagra and of sprue or pernicious anemia (*Ibid.* **1,** *116* (*1943*)) and in distinguishing ocular ariboflavinosis from other conditions involving corneal vascularization (*Ibid.* **3,** *219* (*1945*)). Many observers have had difficulty in making these

differentiations, however, and in addition a number of cases of cheilosis, said to be indistinguishable from each other, are reported to have responded variously to riboflavin, to pyridoxine, to niacin, to ascorbic acid, and to brewers' yeast (*Ibid.* **1,** *327 (1943);* **3,** *195 (1945)*). As might be anticipated from the difficulties of diagnosis, conflicting estimates as to the incidence of riboflavin deficiency have appeared (*Ibid.* **2,** *83, 92 (1944)*). In experimental animals, in addition to dermatitis and eye changes (including cataract formation as well as keratitis), riboflavin deficiency leads to degeneration of nerve tissue (*Ibid.* **1,** *45 (1942)*), anemia (*Ibid.* **1,** *357 (1943)*; **2,** *235 (1944)*), and congenital malformations in the offspring (*Ibid.* **2,** *297 (1944)*).

Very little data have been presented on which to base calculations of the riboflavin requirement of man. This requirement was at first estimated to be approximately 3 mg. per day. The smaller figure of 2 mg. per day was accepted by the Food and Drug Administration and even this is considered to be too high by many investigators. In rats, the requirement is increased somewhat by high levels of dietary fat (*Nutrition Reviews* **3,** *150 (1945)*). Riboflavin appears to have several functions in the animal body; it is a constituent of a number of enzymes including amino acid oxidase, xanthine oxidase, and succinic dehydrogenase (*Ibid.* **3,** *291 (1945)*) as well as Warburg's "yellow enzyme" which has been shown to assist in the oxidation of hexose-phosphoric acid.

The recommended "allowances" for riboflavin were originally placed at approximately one and a half times the values for thiamine or, roughly, 0.9 mg. per 1000 calories (*Nutrition Reviews* **1,** *164 (1943)*). These figures were recently reduced to about 0.6 to 0.7 mg. per 1000 calories (*Ibid.* **3,** *287 (1945)*).

Following the demonstration by C. A. Elvehjem and co-workers that the administration of niacin cures blacktongue in dogs (*Nutrition Reviews* **1,** *68 (1943)*), this vitamin rapidly attained its present position in the treatment of pellagra. In conformity with the rule that deficiencies seldom occur singly, pellagrous patients, it is true, often exhibit signs attributable to the lack of vitamins other than niacin. Nevertheless, the dermatitis, diarrhea, dementia, stomatitis, and glossitis observed in these patients respond, often spectacularly, to niacin therapy and this vitamin thoroughly deserves the title, coined by Goldberger, of the "pellagra-preventive (or P-P) factor."

The enigma of pellagra however has not been completely solved. There remain a number of important and interesting problems, chief among them being the exact relation of the use of corn as a staple food to the appearance of pellagra as an endemic disease. A confusing observation in this connection was that corn eaters developed pellagra on diets containing larger amounts of niacin than were found in other non-pellagra producing diets. New light has been thrown on this question by the discovery by C. A. Elvehjem and his associates that under defined experimental conditions niacin and tryptophane may interchangeably cause stimulation of growth in the rat (*Nutrition Reviews* **3,** *267 (1945)*). The basis of this interchangeability is not clear but if the observation is one of general import it appears to be the first step in the elucidation of the corn-pellagra relationship. Since the tryptophane content of the proteins of corn is low, pellagra as usually encountered may represent a combined deficiency of tryptophane and niacin.

Anemia of a macrocytic type has frequently been observed in patients with pellagra (*Nutrition Reviews* **3,** *154 (1945)*) and has been reported in patients with "parasprue," a deficiency state said to be corrected by niacin therapy (*Ibid.* **3,** *181 (1945)*). In addition, dogs on a diet deficient in niacin may, by treatment with saline, be caused to recover from an acute episode of blacktongue (or on certain purified diets may fail to develop blacktongue as expected) and may

later develop a severe anemia (*Ibid.* **1,** *368 (1943)*). The mechanism by which a niacin deficiency leads to anemia is not as yet understood. The picture is further complicated by the recent report of T. D. Spies and associates (*Ibid.* **4,** *11 (1946)*) that the macrocytic anemia of pellagra, in common with other macrocytic anemias, responds to therapy with synthetic L. casei factor.

A new approach to the evaluation of niacin nutrition has been based on the observations of V. A. Najjar, R. W. Wood, and L. E. Holt, that extraction of alkaline urine by butanol yields a fluorescent solution and that the intensity of the fluorescence is related to the prior ingestion of niacin or niacinamide (*Nutrition Reviews* **2,** *234 (1944)*). The metabolite, designated "F_2," was later identified by J. W. Huff and W. A. Perlzweig as N-methylnicotinamide. A number of investigators agree that the excretion of this compound is decreased in pellagra and that the nutritional state of an individual influences the amount excreted after a test dose of nicotinamide (*Ibid.* **3,** *233, 263 (1945)*). The precise value of measurements of F_2 as a means of assessing the status of niacin nutrition however remains problematic. The study of this problem is being continued in several laboratories.

A precise figure for the human niacin requirement has never been adopted although a value of 10 mg. per person per day has sometimes been employed. In view of the suggested tryptophane-niacin relationship, it appears that in any statement of niacin requirement the protein, or rather amino acid content of the diet will need to be considered. The recommended "allowances" originally adopted were ten times those of thiamine (*Nutrition Reviews* **1,** *164 (1943)*). In the recent revision (*Ibid.* **3,** *287 (1945)*) this ratio has been preserved.

THE VITAMIN COMPOSITION OF HUMAN MILK

Careful studies of the composition of human milk are of interest to pediatricians, obstetricians, nutritionists, biochemists, and physiologists. Such studies bear indirectly on the nutritive requirements of infants and of lactating women. One must beware, however, of supposing that a knowledge of the composition of human milk will give a direct indication of the ideal diet for the infant. It is well known that nature, in "planning" the composition of milk, did not provide for the infant's requirement of iron. There may be other errors in nature's formula.

One might cite a number of isolated examples of the secretion of milk which was deficient in essential dietary factors as a result of a grossly deficient intake by the mothers. There is little information, however, concerning the effect on the composition of milk of a more abundant as contrasted with a less abundant but adequate maternal diet. As a consequence it has been difficult to secure a valid picture of the vitamin content of the milk from healthy mothers at various periods of lactation.

A series of detailed studies which provide a unified picture of the vitamin content of human milk for the first year of lactation has appeared from the laboratories of the Children's Fund of Michigan and cooperating institutions (I. G. Macy, H. H. Williams, J. P. Pratt, and B. M. Hamil, *Am. J. Dis. Child.* **70,** *135 (1945)*; M. Kaucher *et al.*, *Ibid.* **70,** *142 (1945)*; V. Davies, *Ibid.* **70,** *148 (1945)*; M. N. Coryell *et al.*, *Ibid.* **70,** *150 (1945)*; C. E. Roderuck, H. H. Williams, and I. G. Macy, *Ibid.* **70,** *162 (1945)*; C. E. Roderuck, M. N. Coryell, H. H. Williams, and I. G. Macy, *Ibid.* **70,** *171 (1945)*; B. Munks, A. Robinson, H. H. Williams, and I. G. Macy, *Ibid.* **70,** *176 (1945)*; M. Lesher, J. K. Brody, H. H. Williams, and I. G. Macy, *Ibid.* **70,** *182 (1945)*). These papers

summarize the general program of investigation, the dietary control of one group of subjects, the method of obtaining the samples, the technics of analyses, the results, and, finally, the existing data on the comparative value of bovine and human milks in infant feeding (J. M. Lawrence, B. L. Herrington, and L. A. Maynard, *Ibid.* **70,** *193 (1945)*).

In the introductory paper the investigators review reports which emphasize that the breast-fed child "enjoys a better chance to live and to have fewer illnesses," that there are definite psychologic advantages to breast feeding for both mother and child, that instances of "abnormal" breast milk which contradicts nursing are very rare indeed, and that some 87 per cent of mothers can, by proper management, produce a sufficient milk supply to justify continuation of nursing. Pediatricians generally will agree with the authors' viewpoint. Some obstetricians may point out that certain risks to the mother, such as mastitis, are associated with lactation and that these have not been weighed by the authors in this consideration.

The purpose of the present reports is to record the vitamin content of carefully collected samples of milk from a group of healthy mothers. Future reports are to deal with the proximate composition of the milk, metabolic phases of the study of the mothers, and the growth and skeletal maturation of the infants.

The analyzed milk samples were obtained from two groups of women: (a) 70 subjects in the Detroit area who were successfully nursing their infants and who were subsisting on a self-chosen diet. The economic level represented is not stated; (b) 14 women receiving controlled diets of comparable quality. One hundred and eighty-nine samples representing four to eight hour periods were obtained from the first group; 175 twenty-four hour specimens were collected from the second group from the initiation of lactation and at intervals thereafter through the tenth month.

The menu for the group of subjects which was maintained on controlled dietaries during the periods of milk collection is reported. It was different for each of five days and was composed of simple foods "served in a variety of ways to make attractive palatable meals." A generous quantity of dairy products was included. Each day's menu was well distributed throughout the "Seven Basic Food Groups." Weighed duplicate meals were analyzed in order to determine accurately the actual intake of the various food factors. A tabulation of the daily intake as established by analyses of food eaten reveals mean intakes as follows: 2936 calories, 109.5 g. of protein (77 per cent animal), 125 mg. of ascorbic acid, 1455 micrograms of vitamin A, 6248 micrograms of carotene, 1.23 mg. of thiamine, 3.14 mg. of riboflavin, and 16.5 mg. of niacin. It is to be noted that these values closely approximate the revised Recommended Dietary Allowances (*Nutrition Reviews* **3,** *287 (1945)*). The intakes of thiamine, ascorbic acid, and niacin were slightly lower than the recommended allowance. The evidence is convincing that these subjects were abundantly fed.

The milk samples were expressed manually by a simple technic devised by V. Davies (*loc. cit.*). It should be noted that this technic is applicable in many conditions (malformed nipples, failure of the infant to suckle, and the like) which permanently or temporarily prevent the mother from nursing her infant, and its use in these cases would make mother's milk available to the infant in such circumstances. The sterile, measured samples were either analyzed at once or were held at −50°F. until analyses for B-vitamins were made. The remainder was then dried in a frozen state by the cryochem process (D. M. Teague *et al., J. Lab. Clin. Med.* **28,** *343 (1942)*) and stored for future determination of proximate composition.

The methods of assay for the B-vitamins are reported in detail. Niacin, pantothenic

acid, and biotin were measured by microbiologic procedures employing L. arabinosus, L. casei, and L. arabinosus respectively. Total and free thiamine were estimated by a thiochrome procedure. Total thiamine was determined after digestion with clarase; the free thiamine was that found without prior digestion. The riboflavin was measured fluorometrically and it was also divided into free and combined fractions. The ascorbic acid estimations included measures of both oxidized (dehydro) and reduced ascorbic acid. Vitamin A measurements were made by the Carr-Price reaction and internal standards were employed. The carotenes were expressed in terms of a beta-carotene standard. The results are reported for the concentration of all these substances in the milk secreted by the group of women receiving known diets throughout the first ten postpartum days. The later data on this same group and on the random samples from the women consuming self-chosen dietaries are reported together.

A review of the charts impresses one with the adjustments which occur in the concentrations of vitamins in the milk during the first ten days and then the relatively constant mean concentrations which were observed throughout the remainder of the period of study. There was great variation in the concentration of all factors measured in the mature milk samples and this variability was not greatly reduced by controlling the diet of the woman. It is true that the extremes of the observed ranges were greater in the uncontrolled group, but this is to be expected since there were more observations made on the group eating the self-chosen diet. The concentration of niacin, pantothenic acid, biotin, and riboflavin in colostrum was low during the first three to eight days postpartum. By the tenth day these factors had attained approximately their mature levels, the means of which are indicated in the accompanying table. Thiamine, both total and free, increased more slowly and by the tenth postpartum day had reached only about one-half the concentration which was observed in the mature milk. In contrast, ascorbic acid was found to be secreted into the earliest colostrum at approximately the same concentration as in mature milk. Finally, the fat soluble vitamin A and carotene were in relatively high concentrations in the early colostrum and these factors decrease to a stable level of one-half to one sixth of their initial concentrations in mature milk. This finding recalls similar observations which have been made on bovine colostrum and vitamin A (*Nutrition Reviews* **1**, *206* (*1943*)).

TABLE

Average Composition of Mature Human Milk

FACTOR	WOMEN ON KNOWN DIET		WOMEN ON SELF-CHOSEN DIET	
	Mean	*S.D.*	*Mean*	*S.D.*
Niacin, micrograms per 100 ml	196	38.2	176	51.3
Calcium pantothenate, micrograms per 100 ml	256	44.3	242	69.9
Biotin, "	0.80	0.484	0.82	0.490
Thiamine, total, "	14.8	—	14.0	—
Thiamine, free, "	6.67	—	4.66	—
Riboflavin, total, "	41.3	—	35.4	—
Riboflavin, free	28.4	—	22.3	—
Ascorbic acid, micrograms per 100 ml	5.7–7.0	—	3.6–5.5	—
Vitamin A, "	62	—	60	—
Carotenoids, "	23.9	—	25.5	—

The average values of mature human milk as obtained in this study are summarized in the table. When one recalls that the total milk output may rise to 1000

ml. per day by the fourth month of lactation, the indicated loss of vitamins by the maternal organism by way of the milk is striking.

It is worthy of comment that the mean values obtained for the milk samples from the women on self-selected dietaries were not greatly different from the means of the data from women who were known to partake of abundant diets during the period of milk collection. Where differences existed the trend was for slightly lower concentrations of the vitamins in the group on self-chosen regimens. It is not possible to conclude from the data whether this tendency is a reflection of dietary differences or (more probably?) merely a result of collecting the samples at various times of day rather than throughout the twenty-four hour period.

These comprehensive studies are important contributions to our knowledge of the vitamin composition of human milk which has been produced by adequately nourished mothers and which has proved satisfactory for infant feeding. An important part of these reports is the critical summary which they provide of the literature of similar studies. The remaining papers of this series will be awaited by all investigators interested in the nutrition of infants and of childbearing women.

CEROID PIGMENT IN DIETARY CIRRHOSIS OF RATS

R. D. Lillie, F. S. Daft, and W. H. Sebrell (*Pub. Health Reports* **56,** *1255 (1941)*) described a yellow, fluorescent, acid fast "ceroid" pigment which was present in great amounts in the livers of rats in which cirrhosis was produced by dietary means. The cause and nature of this pigment have attracted the attention of a number of investigators. Nevertheless the precise factors which condition its appearance are as yet unknown. It has been found that dietary cirrhosis can be produced without these pigmentary deposits (K. M. Endicott, F. S. Daft, and W. H. Sebrell, *Proc. Soc. Exp. Biol. Med.* **57,** *330 (1944)*). Evidence has been presented that cod liver oil may be transformed by oxidation *in vitro* into a fluorescent acid fast material (K. M. Endicott, *Arch. Path.* **37,** *49 (1944)*) and that cod liver oil injected subcutaneously acquires similar properties. This finding would suggest that the oil component of the experimental diets may have provided a source for the pigment. However, M. Wachstein (*Proc. Soc. Exp. Biol. Med.* **59,** *73 (1945)*) has shown that ceroid may be formed even when cod liver oil is omitted from the diet.

Since it is known that vitamin E deficiency in female rats produces a brown coloration of the uterine muscle and since this pigment, like ceroid, resists decolorization with acids after staining with carbolfuchsin, J. Victor and A. M. Pappenheimer (*J. Exp. Med.* **82,** *375 (1945)*) were led to study the relation of this vitamin to the ceroid pigments. Bearing out the suggestion that the pigment observed in vitamin E deficiency and the ceroid pigment of dietary cirrhosis may be related is the observation of H. Dam and K. E. Mason (*Federation Proceedings* **4,** *153 (1945)*) that a diet containing 20 per cent cod liver oil brings about the deposit of acid fast pigment globules in the adipose tissue of young rats and that the pigmentary changes are prevented by the administration of alpha-tocopherol. It is of interest, furthermore, that most of the diets which have been used in the production of nutritional cirrhosis have been deficient in vitamin E. Those that have contained adequate amounts of this vitamin have led to the formation of relatively small amounts of ceroid. Thus when K. M. Endicott, F. S. Daft, and W. H. Sebrell (*Proc. Soc. Exp. Biol. Med.* **57,** *330 (1944)*) included

alpha-tocopherol in their experimental diet, cirrhosis without ceroid was produced.

In the study of Victor and Pappenheimer (*loc. cit.*), a series of 24 rats received a low protein (6 per cent casein) diet, 13 of these receiving alpha-tocopherol, and the remaining 11 serving as controls. The ceroid pigment was absent in all but one of thirteen liver biopsy specimens obtained from the twenty-seventh to the fifty-seventh day from the treated animals, while ceroid was present in 8 of the 11 untreated controls. Strangely, however, this apparent protective effect was not permanent. Although the amount of pigment in the rats receiving tocopherol was always slightly less than in the untreated animals, after one hundred and sixty days ceroid was also found in the livers of the alpha-tocopherol treated rats. Fatty changes and cirrhosis were similar in both groups of animals but there was a striking difference in the general appearance and growth of the treated animals as compared with the controls. The weight curves of the two groups were similar for four months. From this time on the untreated rats declined in weight and there was a marked reduction in food consumption as well as a diffuse thinning of the hair. At the same time the animals became dirty and showed a peculiar orange brown scurf. The animals receiving alpha-tocopherol, on the other hand, grew satisfactorily and were normal in appearance despite having cirrhosis.

Since it has been observed that excess dietary l-cystine produces cirrhosis unaccompanied by fat deposition (D. P. Earle and J. Victor, *J. Exp. Med.* **75,** *179 (1942)*), experiments were carried out in which one group of animals received a low protein diet while the other was given a stock diet. Both groups were given 5 per cent cystine. On the stock diet containing 5 per cent cystine, two of fifteen livers had small amounts of ceroid in a few histiocytes within the periportal scars. In contrast, 11 of 14 rats on the 5 per cent l-cystine low protein diet developed considerable ceroid within histiocytes and liver cells.

In another series of animals alpha-tocopherol was added to the l-cystine low protein diet. It was found that this vitamin did not prevent but, nevertheless, strikingly inhibited the accumulation of ceroid pigment in the liver. Whereas in the alpha-tocopherol treated rats ceroid was present in very small amounts and was confined to a few histiocytes and liver cells, the pigment was abundantly present in the untreated controls. The alpha-tocopherol had no influence on the onset of the cirrhotic changes produced by the 5 per cent l-cystine low protein diet.

Since choline prevents fat accumulation in the liver cells and since most of the diets leading to nutritional cirrhosis with ceroid pigmentation also brought about the formation of large quantities of fat, an experiment was carried out to determine whether fatty change was a necessary precursor to the ceroid. Choline was added to the diet of 15 rats receiving the 5 per cent l-cystine, low protein diet. It was found that only 3 of the 15 had ceroid in the liver. Thus choline retarded and inhibited, though incompletely, the deposition of the ceroid pigment. The data were insufficient to determine whether the inhibitory effect of the choline upon ceroid deposition was due to a direct influence upon its production or acted indirectly by preventing the accumulation of some lipid precursor.

Thus by the use of a 5 per cent l-cystine, low protein diet which apparently speeds up the formation and deposition of ceroid pigment, it has been shown that vitamin E in the form of alpha-tocopherol retards but does not permanently inhibit the appearance of this pigment in the livers of rats on low protein diets; furthermore the presence of pigment apparently is not dependent either upon fatty changes in the liver cells or upon cirrhosis. Victor and Pappenheimer state that they have repeatedly seen extremely

fatty livers without ceroid and, conversely, ceroid may occur in livers with very little fat accumulation.

These workers have observed pigment having similar staining reactions, morphology, and distribution in human cases of cirrhosis and other nutritional disorders and suggest that its occurrence in the tissues may be indicative of an underlying vitamin E deficiency. Although at present this is speculative it is of great interest for, heretofore, no morphologic evidence of vitamin E deficiency in man has been detected. They suggest that when the pigment is present in association with cirrhosis of the liver this may represent an inability of this organ to store or properly metabolize vitamin E brought to it from the intestine. They ask whether the inhibitory effect of alpha-tocopherol may be due to its antioxidant properties or is produced by some more specific chemical reaction, but point out that these and other problems cannot be solved until more is learned of the chemistry of the ceroid pigment and of the biochemical action of the tocopherols.

MALNUTRITION IN EDENTULOUS PATIENTS

The relation of avitaminosis, infection, and a reduction in the vertical dimension of the face to perlèche has been discussed previously (*Nutrition Reviews* **3,** *133 (1945)*). M. Ellenberg and H. Pollack (*J. Am. Med. Assn.* **119,** *790 (1942)*) studied a group of 34 patients with perlèche but no other symptoms of avitaminosis. In 32 cases, the vertical dimension of the face was decreased due to improper dentures. The other 2 patients were not edentulous but had other oral, mechanical defects. Therefore in the 34 cases, the perlèche was due to oral mechanics which provided a proper environment for the development of the lesion, and not due to avitaminosis.

Examples of an interrelationship between proper dentures and malnutrition have been presented by A. W. Mann, J. M. Mann, and T. D. Spies (*J. Am. Dent. Assn.* **32,** *1357 (1945)*) who made a clinical study of 160 edentulous patients with a long standing clinical record of multiple deficiency disease, and who had been under observation for at least three years. All had a previous history or present evidence of chronic pellagra, beriberi, scurvy, and riboflavin deficiency which had been established by the presence of characteristic mucosal membrane lesions of pellagra, of nutritional peripheral neuritis of beriberi, of gingival lesions of scurvy, and of angular cheilosis and ocular lesions of riboflavin deficiency. The vertical dimension of the face was found to be decreased in 140 of the 160 cases. Fifty-seven of the 74 patients who had only one denture or none at all had perlèche. Fifty-two of the 66 patients who were wearing dentures with decreased vertical dimension gave evidence of perlèche. In contrast, only 7 of the 20 cases with a normal vertical dimension showed any angular cheilosis. Ninety-eight of the patients with a reduced vertical dimension complained of symptoms arising from the alimentary tract: "burning of the mouth and tongue, epigastric burning and pain, nausea and vomiting, intermittent diarrhea, cramping and anorexia." Only 8 of the patients with a normal vertical dimension mentioned such symptoms.

In many of these patients, the artificial dentures had been constructed poorly and of inferior materials resulting in much irritation which was augmented by the unsanitary condition of the appliances. Under these circumstances, the consumption of adequate amounts of a varied diet became difficult and often impossible. When patients of this type were given riboflavin, there was a reduction in the cheilotic lesions but at no

time did this treatment result in a disappearance of the lesions if a decreased vertical dimension still existed. Niacin and pyridoxine did not have any effect on angular cheilosis, although they did aid in the reduction of other symptoms.

The data of Mann, Mann, and Spies indicate the necessity to restore adequate dental function by well fitted dentures which restore the normal vertical dimension. However even dentures which are perfect when fitted must be examined periodically to determine whether settling of the alveolar ridges has altered the normal relation. If such a reduction in dental function is allowed to persist, perlèche may result from the decreased vertical dimension of the face, and a complex nutritional deficiency disease may arise from the inability to masticate an adequate amount of food.

RELATIVE NUTRITIVE VALUE OF ANIMAL AND VEGETABLE FATS

A shortage of fat has been perhaps the most distressing dietary problem in this and many previous wars and postwar periods. The wartime shortage has focused attention on the fats and their relative nutritive values. A review by G. R. Cowgill (*Physiol. Rev.* **25,** *664 (1945)*) of the nutritional position of the various fats is therefore timely. In this review the relative worth of fats is discussed from three standpoints: (1) the extent to which they can be digested and absorbed; (2) their content of essential dietary factors (essential fatty acids and vitamins); and (3) their relative value for growth, pregnancy, and lactation. The subjects which have been fully discussed in previous numbers of this journal (*Nutrition Reviews* **1,** *122, 358 (1943)*; **2,** *165, 267 (1944)*; **3,** *271, 332 (1945)*) will be mentioned only briefly here.

The consensus is that in normal adult mammals the most common fats, both animal and vegetable, are absorbed from the intestinal tract to about the same extent (95 to 99 per cent) provided the melting point of the fat is below 50°C. Special highly hydrogenated fats melting at 50°C. or above are progressively less readily absorbed the higher the melting point. In premature and newborn infants, corn and olive oil seem to be more completely absorbed than butterfat (L. E. Holt *et al.*, *J. Pediatrics* **6,** *427 (1935)*; H. C. Tidwell, L. E. Holt, H. L. Farrow, and S. Neale (*Ibid.* **6,** *481 (1935)*).

The situation in regard to the vitamins found in fats may be summarized as follows: vitamin A is found in significant amounts only in butter, egg yolk, and intracellular fats of animal origin. Fish liver oils are the only commercial variety of intracellular animal fats. Vegetable oils, which are usually obtained from seed kernels, are not only devoid of vitamin A itself, but unfortunately contain little or none of the vitamin A precursors (carotenoids). However, this rule finds an important exception in certain oils obtained from the outer seed coverings. A notable example is red palm oil obtained from a palm native to West Africa but now grown in Malaya and Burma. This oil contains nearly 2000 I.U. of provitamin A per gram (U.S.P. cod liver oil contains 600 units of vitamin A per gram) and could, according to estimates, supply a vitamin A source in India, where it is needed, for about one-third its present cost in cod liver oil. Vitamin D is found only in butter and fish liver oils. In England, both vitamins A and D are added to margarine, whereas in this country only vitamin A is added. Vitamin E is found chiefly in seed germ oils. There is little in butter, whereas margarine may contain much or little depending on the types of fats used in its manufacture. The distribution of vitamin K has not been thoroughly studied, but there appears to be little in commercial edible oils. Cowgill outlines the need for determining

the distribution of the essential fatty acids, especially the more active ones, linoleic and arachidonic. Choline is not present in significant quantities in any of the important dietary fats, since the phospholipids which contain choline are largely removed during the refining process.

A major portion of Cowgill's review is devoted to discussing the interesting controversy as to whether butter is superior to other fats for promoting the growth of young animals (*Nutrition Reviews* **2,** *267 (1944)*). There is general agreement that if young animals are ingesting a diet containing carbohydrates other than lactose (or galactose), butter has no advantage over other fats for promoting growth. The controversy arises for the young rat receiving a diet containing lactose, which is, of course, the normal situation for animals drinking milk. In this case some workers have found that butter induces more rapid growth than other fats, whereas other groups of experimenters have failed to find this difference. A suggestion which may rationalize the contradictory results is that butter gives a better growth response than other fats only when the lactose content of the diet is so high that it is detrimental to the animal. That is, butter may in some way help the animal to metabolize lactose. There is evidence that conflicting results by different investigators may arise from a different ability of various animal strains to metabolize lactose. This controversy is pertinent to the problem of the most satisfactory artificial or reconstituted milk for infant feeding in regions of the world with an inadequate supply of cow's milk. It likewise has a bearing on the question of whether other oils should be substituted for butterfat in the nutrition of premature infants as the findings of Holt *et al.* suggest. This question, however, does not seem to be pertinent for the nutrition of older children or adults subsisting on a mixed diet.

Butter and enriched margarine appear to be equivalent in digestibility and caloric contribution to the diet. Butter has a slight advantage over American margarine by virtue of its vitamin D content, and margarine has a similar advantage in regard to vitamin E, which however, is probably a less important contribution.

MICROCHEMICAL METHODS FOR NUTRITIONAL STUDIES

In addition to dietary surveys and clinical studies, determinations made in the laboratory may be of great help in assessing nutritional status. This important area of study is only beginning to receive the attention it deserves. It has not been considered practical to analyze tissues such as blood in surveys because of the size of the samples required. With the advent of reliable microchemical methods, however, we may expect to see the reporting of laboratory findings in more and more nutrition surveys.

In the Public Health Research Institute of the City of New York microchemical methods have been developed which permit the determination of seven nutritive essentials or their corresponding tissue derivatives on two or three drops (0.1 ml. = 100 cu. mm.) of blood (O. H. Lowry and O. A. Bessey, *Federation Proceedings* **4,** *268 (1945)*; *Nutrition Reviews* **3,** *336 (1945)*). These seven essentials are vitamin A, carotene, ascorbic acid, riboflavin, serum protein, hemoglobin, and alkaline phosphatase. Details of the methods and proof of their reliability are being published in a series of papers some of which have appeared and some of which will appear in the near future.

The blood, 0.1 ml., is collected from a finger tip puncture into two capillary tubes, of the type used for melting point determinations. One tube contains heparin; the blood in the other is allowed to clot. The ends of the tubes are sealed with Plicene cement and

sent to the laboratory. Two workers are able to collect about forty specimens an hour. In the laboratory several hundred capillary tubes are centrifuged at a time; they are then broken at the junction between fluid and cellular layers and aliquots removed by Lang-Levy pipettes. These pipettes can be made to deliver volumes of 3 to 1000 cu. mm. with an accuracy of 0.05 to 0.5 per cent.

Vitamin A and carotene are determined in 35 cu. mm. of plasma. Following a microsaponification procedure these nutrients are extracted with a xylene-kerosene mixture and transferred to a microcuvette. Light absorption is read in the Beckman spectrophotometer. Two persons are able to make about one hundred such determinations in a day.

Plasma ascorbic acid is determined on 10 cu. mm. of plasma. The Roe method is used and the color measured with the Beckman spectrophotometer. Two technicians perform about one hundred and fifty determinations per day. Riboflavin is determined in 20 cu. mm. of packed red cells by means of a microbiologic method.

Plasma protein concentrations are calculated from plasma density which is measured with the Lang gradient tube. This ingenious instrument is a liquid column consisting of a mixture of kerosene and brombenzene, so prepared that the density increases progressively from top to bottom. An aqueous drop falls in this calibrated column until it reaches a position of equidensity. The position is read off and the density of the drop ascertained from the calibration figures. After some fifty to seventy determinations in duplicate the plasma droplets are removed by a small amount of sea sand which carries them to the bottom. A team of two people perform sixty to seventy determinations per hour by this method.

At the time of collection of the blood, a 5 to 10 cu. mm. specimen, measured accurately by means of a constriction pipette, is mixed with 1 ml. of dilute ammonia. Hemoglobin concentration is indicated by the color density, which is measured in a photoelectric colorimeter. The error of the method can be as little as 2 or 3 per cent if care is exercised in drawing the blood.

Phosphatase is determined in 3 cu. mm. of serum by means of a newly developed method. P-nitrophenyl phosphate is incubated with the serum in a buffered solution. The degree of hydrolysis of the phosphate, determined by the yellow color of the resulting p-nitrophenol, is a measure of the amount of phosphatase present in the serum. Several hundred determinations per day may be made by two technicians.

These methods have already been applied in a survey in Newfoundland (*Nutrition Reviews* **3,** *251 (1945)*) and in a survey of New York schoolchildren. In the latter study one finding has been that 25 per cent of schoolchildren from a low economic area had plasma ascorbic acid levels below 0.4 mg. per cent while no schoolchildren from a high economic area had similarly low levels. Vitamin A, carotene, and hemoglobin values showed similar distributions. Details of the study have not as yet been published.

As pointed out by Lowry and Bessey, it will be necessary to correlate laboratory findings with dietary intake and with the health of the individuals studied. This could hardly have been considered, however, until accurate micromethods were available. It appears that there is in these technics a tool which promises much as an aid in the determination of the nutritional status of large population groups.

WARTIME DENTAL CARIES INCIDENCE

During World War II, a greatly reduced caries incidence was observed among the children of Norway by G. Toverud (*Munnpleien* **28,** *4 (Sept.) (1945)*). In 1939,

there was an average of 5.6 cavities per child in the 2½ to 3 year age group in contrast to 1.8 cavities in 1944. Among the 6 to 7 year old children, the average number of cavities in 1939 was 29.5 but in 1944 was 19.2. Similar reductions in caries incidence was observed in the children of the other age groups. The consumption of sugar, refined flour, candy, and soft drinks was negligible and the total caloric intake was reduced considerably during the war years. Toverud suggested that this reduction in refined carbohydrate intake was a probable cause of the decrease in dental caries incidence.

M. Mellanby and H. Coumoulos (*Brit. Med. J.* **1**, *837 (1944)*) have reported a comparison between two dental surveys of 5 year old children in the same or comparable schools in London made in 1929 and in 1943. During the intervening period, a great improvement had taken place, both in the structure of the deciduous teeth and in their susceptibility to tooth decay. In 1929 only 8 per cent of the children had teeth of perfect or nearly perfect structure and 58 per cent had defectively formed teeth. However in 1943, 19 per cent of the children had teeth of perfect or nearly perfect structure and only 33 per cent had defectively formed teeth. In 1929 only 5 per cent of the children were caries free while in 1943, 22 per cent were caries free. At both surveys the dental diagnosis was made by oral examination without the aid of radiographs. Mellanby and Coumoulos attributed the improvement in the dentition and the reduction in the rate of tooth decay to the measures which had been taken to improve the nutritional status of these children. Milk had been made available at a reduced cost in the schools. Margarine had been fortified with vitamins A and D, and bread with calcium carbonate. Increased allowances of milk, cod liver oil, and fruit juices had been made available to pregnant and lactating women and to young children. It will be interesting to know if this improvement in dental structure and in resistance to tooth decay will continue when wartime shortages of refined carbohydrate foods are relieved in England. In fact this public health program in future years may help to answer many questions on the relation of nutrition and local environment to dental caries susceptibility.

The relation of nutrition to dental caries incidence has been the subject of great controversy for two or three decades. The two papers reviewed present the two opposing views of the relation of food to dental caries. Toverud interpreted his data to indicate that the reduced caries incidence was due to the reduction in consumption of refined carbohydrates. On the other hand, Mellanby and Coumoulos believed that their data indicated the value of adequate nutrition in the reduction of dental caries susceptibility. The improvement in the structure of the deciduous teeth observed suggested more favorable systemic conditions for tooth development. However the possible relation of the reduction in refined carbohydrates which occurred at approximately the same time in England as in Norway was not considered by Mellanby and Coumoulos. On the other hand Toverud did not discuss the possible relation of nutrition in the reduced caries incidence observed in the Norwegian children. During the war there was an increase in the consumption of coarse grains, unrefined grain products, vegetables, and fish to compensate partially for the decrease in refined foods, candy, and soft drinks. Even though the total caloric intake was lowered, the war diet obtained through rationing may have been more adequate for the development of teeth and their maintenance after eruption than the prewar diet.

The effect of diet through oral environment and through nutrition of the teeth constitutes an entangled problem. The data of Toverud and of Mellanby and Coumoulos are of great interest in the planning of dental health programs.

THE INFLUENCE OF PLASMA LIPIDS ON ELECTROPHORETIC PATTERNS OF HUMAN AND DOG PLASMA

In dogs in which hypoproteinemia was produced experimentally, L. J. Zeldis, E. L. Alling, A. B. McCoord, and J. P. Kulka (*J. Exp. Med.* **82,** *157 (1945)*) observed that there was an apparently paradoxical increase in the areas of the alpha-globulin peaks in the electrophoretic pattern of the plasma. They undertook to determine whether these increases were due to protein or to other materials. For several reasons, the latter seemed likely. For example, nitrogen analyses of the plasma frequently yielded lower values for total protein concentration than those indicated by the electrophoretic areas. Furthermore, increased plasma cholesterol and total plasma lipid levels were regularly found to be associated with these discrepancies. That this difference might be due to elevated plasma lipid levels was also suggested by the observations of L. G. Longsworth and D. A. MacInnes (*J. Exp. Med.* **71,** *77 (1940)*) who reported decreases in the beta-globulin components in the ether extracted plasma of human cases of nephrosis.

L. J. Zeldis, E. L. Alling, A. B. McCoord, and J. P. Kulka (*J. Exp. Med.* **82,** *411 (1945)*) report the electrophoretic patterns of human and dog plasmas before and after extraction of the plasma lipids. The latter were removed by a cold neutral alcohol technic. By this means cholesterol was completely extracted but phospholipids were less thoroughly removed. Presumably the acidic phosphatides, cephalin and phosphatidyl serine, comprised the bulk of the material not removed by the cold neutral alcohol.

In the electrophoretic patterns of normal dog plasma three distinct peaks are usually present between the alpha- and beta-globulin components. In hypoproteinemic dogs such peaks are frequently four in number, the fourth peak often being poorly separated from the beta component. In the extracted plasmas of hypoproteinemic dogs the alpha-1, alpha-2, and alpha-3 components regularly merged into a single peak. In normal dogs the alpha-1 and 2 peaks were found to be merged and the alpha-3 peak apparently emerged with the beta component.

In attempting an explanation of these differences between native and extracted plasmas one must consider the difference produced by the removal of lipids. Another possible explanation, however, is that an incipient denaturation of the proteins had occurred without loss of solubility, thus altering the mobility of the components. Still another possibility is that following the removal of lipids certain of the alpha-globulin components with or without incipient denaturation form complexes with, or are adsorbed on other alpha-globulins or, in the case of the alpha-4 globulin, on the beta component. In human plasmas, however, changes in the alpha-globulin peaks comparable to those seen in the dogs were not observed. This suggests that incipient denaturation is not the explanation of the changes following extraction of the dog plasmas.

In the dog plasmas changes were observed also in the total electrophoretic area and in the areas of individual peaks following extraction of these samples. In general, the total electrophoretic area was reduced as much as 10 to 15 per cent by the extraction of a large part of the plasma lipids in hyperlipemic dogs, a figure which is in agreement with the magnitude of the discrepancies between nitrogen content and electrophoretic concentration. Both the greatest relative and the greatest absolute loss of area regularly occurred in the alpha-globulin peaks as already mentioned and sometimes amounted to 20 or 30 per cent of the original area of these components. Reduction in the area of the beta-globulin was also con-

sistent and averaged about 10 per cent of the original area.

Studies of human plasma were made in a single case each of obstructive jaundice with cholangitis, xanthoma tuberosum, multiple myeloma, nephrosis, and cirrhosis. In the human plasmas large beta-globulin peaks were regularly observed in the presence of elevated plasma lipids. Marked increases in gamma-globulin, however, were also found to be due in large part to elevated plasma lipid levels.

CHRONIC FLUORINE INTOXICATION AND THE SKELETAL SYSTEM

W. H. Bauer (*Am. J. Orth. and Oral Surg.: Sect. Oral Surg.* **31,** *700 (1945)*) has studied the effect of experimental fluoride ingestion on the bones and teeth of dogs. Levels of sodium fluoride varying from 0.01 to 0.08 g. per kilogram of body weight were given daily for periods of twenty-nine to one hundred and twenty-eight days. Grossly, a striking thickening was observed in the bones of those dogs which had received fluorine. The surfaces of the bones were rough, uneven, and chalky. The long bones, jaws and vertebrae were most frequently and most severely affected. Transverse sections of the bones indicated that the thickening was due to a massive periosteal bone formation which was superimposed upon the original, thin cortex. A fairly sharp boundary between the compact and the periosteal bone was maintained. A distinct osteoporosis was observed in the spongy regions of bone. The consistency of the bones varied from brittle in those dogs which had received the least fluorine to soft and pliable in those which had received the most. Radiographic examination of the jaws containing the permanent tooth buds demonstrated not only thickening of the jaws but distinctive changes in the hard structure of the tooth buds. The eruption of the permanent teeth was delayed in all dogs which had received fluorine. All teeth developed during the period of fluorine administration, especially the premolars and the molars, had brownish, discolored areas of enamel hypoplasias around the cusps. The enamel in these regions was soft and easily chipped.

The earliest changes observed in the bones were in puppies which had been fed the milk of their fluorine-poisoned mother exclusively for twenty-eight days. Even after this short period, the long bones and jaws exhibited remarkable periosteal bone deposition at the insertion of the muscles and tendons. It was interesting to note that in the tooth buds of the 2 puppies sacrificed at twenty-eight days no changes were observed. Thus in these cases the bone changes had preceded the tooth pathology.

Reports on the relation of chronic fluorine intoxication to mottled enamel in man have been much more frequent than those concerned with bone pathology. H. E. Shortt, G. R. McRobert, T. W. Barnard, and A. S. M. Nayar (*Indian J. Med. Res.* **25,** *553 (1937)*) observed a large number of cases of skeletal involvement in districts of the Madras presidency where there was a high incidence of severely mottled enamel. Ten cases were selected for detailed clinical, radiologic, and biochemical study. A definite rigidity of the entire vertebral column was present, especially in the lumbar region. Frequently neither anteroposterior nor lateral movement was possible. Definite bony outgrowths were observed at the insertions of tendons to the long bones. The edges of the ribs were irregular due to bony outgrowths. A definite limitation of movement was observed at all joints and movements of the joints were accompanied by intense pain. The chest was almost immobile due to the fixation of the ribs; breathing in some cases was entirely abdominal. Radiologic examination revealed an increased density of the

vertebrae, ribs, pelvis, and long bones. Estimations of serum calcium, inorganic phosphorus, and serum phosphatase indicated favorable conditions for the deposition of bone.

C. G. Pandit, T. N. S. Raghavachari, D. Subba Rao, and V. Krishnamurti (*Indian J. Med. Res.* **28,** *533 (1940)*) observed that the severe forms of chronic fluorine intoxication described by Shortt *et al.* were restricted to three districts in northern Nellore of the Madras presidency. The fluorine in the drinking water was present in a concentration of 2.5 parts per million or more. Continued residence of over fifteen years was necessary in adults to produce the symptoms. Pandit *et al.* observed some interesting relations between the nutrition of the people in a district and the susceptibility to this disease. A pronounced deficiency of vitamin C was associated with a severe incidence of these pathologic changes in the bone. Similarly an increased intake of calcium seemed to result in an increased susceptibility. The people in the lower economic groups appeared to be more susceptible.

In South Africa, T. Ockerse (*South African Med. J.* **15,** *261 (1941)*) observed 6 cases of chronic fluorine intoxication at a farm where the drinking water contained 11.78 p.p.m. of fluorine. The symptoms and radiologic description presented were identical with those described by Shortt *et al.* As far as is known only one case of chronic fluorine intoxication with bone pathology has been described in man in United States. J. F. Linsman and C. A. McMurray (*Radiology* **40,** *474 (1943)*) reported a case of osteosclerosis exhibiting severe mottled enamel. The diagnosis of fluorine toxicosis was proved by the history of almost continuous residence in regions of Texas at which the communal water supplies varied from 4.4 to 12 p.p.m.

No reports of studies of bone and teeth simultaneously in growing children who resided in fluorine areas were available until F. H. Kemp, M. M. Murray, and D. C. Wilson (*Lancet* **II,** *93 (1942)*) examined 22 children, from 9 to 14½ years of age, with moderate to severe dental fluorosis. Sixteen of the children were from districts where the water supply contained from 0.3 to 1.2 p.p.m. of fluorine. The other 6 were from a district where the fluorine concentration in the drinking water was 5.0 p.p.m. These studies were limited to an examination of the vertebrae. The findings suggested that disturbances in the natural processes of calcification and bone formation were relatively common in children, but that there was not necessarily any relation between fluorine and these disturbances. However, 2 of the cases from the lower fluorine districts which had severe dental fluorosis had skeletal changes and the 6 cases from the high fluorine district had severe mottled enamel and disturbances of ossification. Kemp *et al.* felt that there was a higher incidence of bone changes when the diet was poor than when it was adequate.

These studies are of particular importance now when the relation of fluorine to the maintenance of dental health is being widely discussed. The studies of Shortt *et al.* and Pandit and co-workers in India, Ockerse in South Africa, and Linsman and McMurray in this country were made in regions where the fluorine content of the drinking water was in excess of 2.5 p.p.m. Such high levels have been recognized to be excessive for the development of normal teeth for some years. However the investigation of Kemp *et al.* was partially made in regions where the fluorine concentration of the drinking water varied between 0.3 and 1.2 p.p.m. A more detailed, extensive examination of large numbers of individuals in all age groups in these districts would be of great value. A simultaneous nutritional investigation of the relation of specific deficiencies to the pathologic conditions of the skeletal system would add much to our knowledge of the relation of fluorine to tooth and bone development.

One point in Bauer's studies on chronic fluorine intoxication in dogs which needs further, careful investigation is the relative susceptibility of bone formation and tooth development to pathologic changes. In the 2 puppies which received only milk from their fluorine-poisoned mother the bone changes occurred before any alteration in the developing tooth buds. This would indicate that the bone disturbances occurred more readily than those in the developing teeth. If this observation should be substantiated, it would indicate that long exposure to low levels of fluorine might produce some pathologic changes in the skeletal system.

CARBOHYDRATE ABSORPTION IN SPRUE

According to F. Verzár ("Absorption from the Intestine," *Longmans, London (1936)*), dextrose is absorbed mainly after phosphorylation has taken place, whereas fructose passes through the wall of the alimentary canal chiefly by a process of simple diffusion. It is therefore of importance to know whether there is any difference in the absorption of these two sugars in diseases of the gastrointestinal tract, where this process might be impaired. B. G. Maegraith and co-workers (*Lancet* **II,** *635 (1945)*) have made a preliminary report on the comparative rates of absorption of dextrose and fructose in patients suffering from sprue. The subjects were mostly native Indian troops who had been in action on the India-Burma frontier. All had a history of fatty diarrhea, dyspepsia, loss of weight, anemia, and "some degree of vitamin deficiency." Most were in the stage of convalescence when studied, although some were still in the acute stage. An effort was made to carry out the observations early in the period of hospitalization, usually within the first week before any vitamin or liver therapy had been instituted, and while the patient was on a simple milk diet. The absorption or tolerance test consisted of the ingestion of 100 g. of sucrose at least twelve hours after the last food had been taken, and the estimation of blood levels of dextrose and fructose every half hour for two and a half hours. The findings compare favorably with those which have been reported previously (G. R. Cowgill, "The Vitamin B Requirement of Man," *Yale University Press, New Haven (1934)*; J. Groen, *New Engl. J. Med.* **218,** *247 (1938)*). The dextrose absorption curve was flat with a peak seldom more than 20 mg. per 100 ml. higher than the fasting control level. No intravenous tolerance tests were done but it has been established that in this condition the flat curve indicates an absorption defect rather than rapid utilization, storage, or excretion. The fructose absorption curve was within the normal range. Follow-up observations during convalescence when an increase in the diet had been effected, demonstrated a slow improvement in the dextrose absorption curve which finally became normal. There was no change in the normal fructose tolerance curve. The abnormal absorption during the active phase of sprue was interpreted by these workers as meaning that the diffusion of hexoses through the gut membrane was normal but the active absorption of dextrose, presumably dependent upon phosphorylation, was greatly impeded. Thus they surmise that in sprue a phosphorylation defect may be a characteristic "physiological lesion."

Since *in vitro* studies have shown that magnesium has an important function in phosphorylation, plasma magnesium and calcium levels were investigated at the same time that carbohydrate absorption was determined. Even where the magnesium levels were low and clinical tetany occurred, there was no parallelism between absorption of carbohydrate and plasma levels of magnesium or calcium. Indeed, "no direct

relationship was observed between the recovery of the carbohydrate absorption and the return of magnesium levels to normal." Failure to find any correlation between magnesium level in plasma and the inhibition of absorption of dextrose was not discussed.

IMPROVEMENTS IN ASCORBIC ACID AND THIAMINE DETERMINATIONS

Modifications of ascorbic acid and thiamine analytical procedures continue to provide greater specificity and usefulness. For ascorbic acid, O. H. Lowry, J. A. Lopez, and O. A. Bessey (*J. Biol. Chem.* **160,** *609 (1945)*) have developed a sensitive micromethod for determination in as little as 0.01 ml. of blood serum. This procedure is but one designed for use in nutritional survey work in which several types of determinations may be made after the convenient collection of approximately 0.1 ml. of blood obtained by simple finger puncture.

For this particular analysis, the dinitrophenylhydrazine procedure of J. H. Roe and C. A. Kuether (*J. Biol. Chem.* **147,** *399 (1943)*) was adapted in several ways for special micro aspects. There was a reduction in the relative quantity of charcoal used for oxidation of the ascorbic acid, and this was used as a suspension in trichloracetic acid instead of dry. Removal of charcoal and precipitated proteins were accomplished in one step, and the thiourea and dinitrophenylhydrazine reagents were combined. Final color measurements were made with a Beckman spectrophotometer equipped with a special diaphragm, and in microcuvettes.

Of particular convenience to the analyst was the finding that the acidified filtrate retained its level of ascorbic acid for six days at room temperature and thirteen days at 4°C. Comparison of the method with the macro procedure of R. L. Mindlin and A. M. Butler (*J. Biol. Chem.* **122,** *673 (1938)*) showed good agreement. It is claimed that one person can analyze fifty sera in one day.

Turning to the determination of ascorbic acid in food products, W. B. Robinson and E. Stotz (*J. Biol. Chem.* **160,** *217 (1945)*) claimed to have increased the specificity of the indophenol method. Their basic method involves the extraction of the excess indophenol dye after reaction with ascorbic acid by xylene, and measurement of color in this solvent in a photoelectric colorimeter. The basic principle of this procedure was described by F. Bukatsch (*Z. f. physiol. Chem.* **262,** *20 (1939)*) and further developed by E. Stotz (*J. Lab. Clin. Med.* **26,** *1542 (1941)*) and L. P. Pepkowitz (*J. Biol. Chem.* **151,** *405 (1943)*), and in itself eliminates the interference of many pigments in food products.

The interfering reducing action of sulfites, and of stannous and ferrous salts, which may occur in sulfite treated dehydrated foods and canned acid products, respectively, is eliminated by the addition of hydrogen peroxide prior to dye addition. The oxidizing action of the added peroxide on leuco dye, which may be serious in a titrimetric procedure, is obviated by the quick removal of dye from the reaction mixture in the xylene extraction procedure. The extent of interference of "reductones" was accounted for by preliminary condensation of the true ascorbic acid with formaldehyde. In the latter test "reductone" prepared by the action of alkali on glucose, and "reductinic acid" resulting from the reaction of strong acid on pentose, were not completely differentiated from ascorbic acid by formaldehyde. There is no evidence, however, that these are the actual substances which occur in stored food products, while the actual nonascorbic acid reducing material in stored dehydrated cabbage was found to be completely distinguishable from ascorbic acid in the formaldehyde procedure. Several examples are given of the false ascorbic acid values which may be obtained by direct reaction of the acid fil-

trate of food products with indophenol dye, and it is advised to ascertain initially whether nonascorbic acid substances occur in a given food product before routine analysis.

Some doubt has been cast on the specificity of the dinitrophenylhydrazine procedure for dehydroascorbic acid by the experiments of M. Pijoan and H. J. Gerjovich (*Science* **103,** *202* (*1946*)). The observation was made that an aerated orange juice which appeared to contain 60 to 70 mg. per cent of dehydroascorbic acid by the dinitrophenylhydrazine method did not protect guinea pigs from scurvy, and that the hydrogen sulfide reduction method revealed only 4 mg. per cent. Attention is called to the fact that diketogulonic acid, a further breakdown product of dehydroascorbic acid, also reacts with dinitrophenylhydrazine (J. R. Penney and S. S. Zilva, *Biochem. J.* **37,** *39* (*1943*)). Caution in applying the method to tissues which have not been as thoroughly tested as blood is advised by Pijoan and Gerjovich.

The uncertainty of dehydroascorbic acid determinations exists at the same time that increasing demands are made for such analyses. Employing the dinitrophenylhydrazine procedure, T. J. McMillan and E. N. Todhunter (*Science* **103,** *196* (*1946*)) have found, for example, that 50 per cent of the total ascorbic acid in cabbage that had been cooked and held on the steam table for two hours, reacted as dehydroascorbic acid.

Toward a better thiochrome procedure for thiamine estimation in urine, O. Mickelsen, H. Condiff, and A. Keys (*J. Biol. Chem.* **160,** *361* (*1945*)) have made a systematic study of the extraction of thiochrome by isobutanol. In the usual procedures, the eluate from permutit still contains interfering substances which apparently yield fluorescent products on ferricyanide oxidation, and are furthermore extracted by isobutanol. Adjustment of the pH to 8 to 9.5 before extraction of the thiochrome appears to minimize extraction of these substances. Using urines from which recovery values of small amounts of thiamine by the older method were zero, from 91 to 103 per cent recoveries were obtained by making the pH adjustment of the final aqueous solution. The new modification, unlike older procedures, never yields negative values, and produces theoretical results in mixtures of urines which have been separately analyzed. Although there is still no final criterion by which a thiamine estimation in urine can be checked, there can be no doubt that the method described does eliminate some errors frequently occurring in the most commonly used procedures.

A rapid method of estimating thiamine in urine by the thiochrome procedure was devised by W. A. Perlzweig, H. Kamin, I. Gue, and J. V. Blalock (*Arch. Biochem.* **6,** *97* (*1945*)). In this method time is saved by eliminating the adsorption column and carrying out the adsorption and elution in centrifuge tubes, in the way described by A. D. Emmett, G. Peacock, and R. A. Brown (*J. Biol. Chem.* **135,** *131* (*1940*)). The usual elution is replaced by an acid pyridine-methanol mixture, and the final extraction from the aqueous phase is made by n-butanol instead of by iso-butanol. This modification is rapid and good precision is claimed for it.

THE EFFECT OF CHLORIDE DEFICIENCY ON RATS

E. Orent-Keiles, A. Robinson, and E. V. McCollum (*Am. J. Physiol.* **119,** *651* (*1937*)) restricted rats to a diet very low in chloride but of unknown chlorine content, and during a ninety day period of observation noted a retardation of growth but no other symptoms. D. M. Greenberg and E. M. Cuthbertson (*J. Biol. Chem.* **145,** *179* (*1942*)) induced chloride deficiency with a diet containing only 0.012 per cent of chlorine and

noted that when fed to rats it caused a reduction in growth rate and in blood chloride, an increased blood bicarbonate, and a reduction in urinary chloride almost to zero. L. Voris and E. J. Thacker (*J. Nutrition* **23,** *365 (1942)*) and E. J. Thacker (*Ibid.* **26,** *431 (1943)*) used a diet containing 0.02 per cent of chlorine and studied energy and protein metabolism and the mineral composition of the body. The chloride deficient rats lost more energy as heat compared with pair-fed controls, and consequently stored less as tissue. In the entire body there was a decrease in the content of chloride, sodium, and potassium, and an increase in the content of calcium, magnesium, and phosphorus, probably because the ratio of soft tissue to skeleton was decreased.

E. M. Cuthbertson and D. M. Greenberg (*J. Biol. Chem.* **160,** *83 (1945)*) have now investigated more closely the physiologic disturbances caused by deficiency of chloride because they noted that chloride was the only one of the major mineral constituents of the body for which proof was lacking that its dietary deficiency leads to pathologic manifestations. An exceptionally small amount of chloride was present in their experimental diet. It had only 0.002 to 0.005 per cent of halide, and a total carbonate and bicarbonate content of 2 per cent, while the control diet had 0.9 per cent carbonate and 0.6 per cent of chloride.

The failure of growth was more striking than had been observed before. The control rats attained a weight of 400 g. at the age of 18 weeks, whereas the deficient rats tended to reach about 130 g. at 12 weeks with no further gain. In groups of pair-fed rats, over a period of ten weeks, the controls grew 171 g. while the chloride deficient rats grew only 73 g. The smaller amount of energy stored as tissue is here strikingly revealed, and the question may be raised whether this is due in part to the excessive work performed by the kidney in order to conserve chloride. The chloride content of all tissues except the heart, spleen, and incisor teeth showed a decrease during the deficiency.

In their earlier work, the authors (*loc. cit.*) had observed that the increase in total carbon dioxide of the blood did not balance the decrease in blood chloride. Total base content of the serum was therefore determined on 12 control and 12 deficient rats, and no decrease was observed, implying an increase in some unknown anion. The excretion of citrate was measured. Control rats excreted 0.44 mg. of citrate daily in contrast to 7.5 mg. for the deficient animals. This agreed with the expectation that the deficient rats showed alkalosis, even though compensated.

Gross examination at autopsy revealed changes suggestive of fatty degeneration, but histologic examination showed a different type of pathologic change. Kidney damage was extensive; degeneration of glomeruli to cyst-like structures with flat epithelium had occurred, the nuclei in these glomeruli were deteriorated, and in some there was complete replacement by scar tissue. There were changes in the epithelial cells of the convoluted tubules, and in the medulla many collecting tubules had degenerated. Increased interstitial tissue was noted in both cortex and medulla, with scar tissue replacing many tubules.

Most of the rats autopsied had been on the deficient diet for eight months, although advanced degeneration was observed in some which had received the deficient diet for only one month. In spite of these changes occurring so early, some of the rats lived on the deficient diet for more than a year.

THE FATE OF TRYPTOPHANE IN MAMMALS

Kynurenine, kynurenic acid and xanthurenic acid are end products of the metabolism of tryptophane which have been detected in various species under certain

conditions. Kynurenine is a tryptophane derivative formed by the opening of the pyrrole ring. Kynurenic acid and xanthurenic acids are products formed from kynurenine by a new (quinoline) ring closure and by oxidation. Xanthurenic acid represents a further stage of oxidation than kynurenic acid. The excretion of these compounds in dogs, normal, pyridoxine deficient, and nicotinic acid deficient, has been studied by H. E. Axelrod, A. F. Morgan, and S. Lepkovsky (*J. Biol. Chem.* **160,** *155 (1945)*).

Previous information in this field was not very extensive. L. Musajo (*Gazz. chim. ital.* **67,** *179 (1937)*) identified xanthurenic acid in the urine of rats and rabbits maintained on a high protein diet, but did not find it in the urine of dogs on a similar type of ration. P. J. Fouts and S. Lepkovsky (*Proc. Soc. Exp. Biol. Med.* **50,** *221 (1942)*) detected a green pigment-producing compound in the urine of dogs on a diet deficient in pyridoxine, and identified it as xanthurenic acid. This compound has also been reported by M. M. Wintrobe *et al.* (*Bull. Johns Hopkins Hosp.* **72,** *1 (1943)*) to be present in the urine of swine rendered deficient in pyridoxine; and the administration of tryptophane to such swine was shown by G. E. Cartwright, M. M. Wintrobe, and S. Humphreys (*J. Biol. Chem.* **153,** *171 (1944)*) to increase the amount excreted.

Young spaniels raised in the laboratory by Axelrod, Morgan, and Lepkovsky were placed on a simplified pyridoxine deficient diet upon reaching a weight of 3 kg. (at 6 to 8 weeks of age). The presence and severity of pyridoxine deficiency were followed by hemoglobin determinations, and qualitative tests for xanthurenic acid, kynurenine, and kynurenic acid in the urine were carried out. It was found to be necessary to test the urine samples shortly after collection, because even when the samples were stored in the refrigerator both xanthurenic and kynurenic acids were unstable.

In the experiments reported 13 pyridoxine deficient and 10 normal control dogs were used. Four deficient and 2 normal dogs received a diet containing 18 per cent of casein, and the remainder received a diet with 45.8 per cent of casein. The normal dogs excreted kynurenic acid generally for two to nine hours after the administration of tryptophane, whereas the pyridoxine deficient animals often excreted xanthurenic acid for twenty-four hours. In general the period of excretion was longer in the more severely deficient dogs. No excretion of xanthurenic acid was observed in deficient dogs on either the low or the high protein diets until tryptophane was given. This finding is in contrast to the case with pyridoxine deficient rats, rabbits, and swine which appear to excrete xanthurenic acid even on high protein diets.

The administration of 2 g. of dl-tryptophane or 1.5 g. of l(−)-tryptophane to severely deficient dogs caused nausea, vomiting, anorexia, and sometimes collapse, but normal or slightly deficient dogs were unaffected. The 18 per cent protein diet led to the production of the same symptoms as the 45.8 per cent protein diet, although they took a somewhat longer time to appear. No dog exhibited deficient symptoms or abnormal excretion (i.e. of xanthurenic acid after dosing with tryptophane) after thirty days on the deficient rations, and on the 18 per cent protein diet no abnormality was noticed after a hundred days, although the number of dogs so tested was small. After a hundred days on the 45.8 per cent protein diet the dogs reacted abnormally to dosage with tryptophane.

Two dogs were maintained on the control diet containing 45.8 per cent casein from which nicotinic acid was omitted, and showed no abnormality; even after several months they metabolized tryptophane to kynurenic acid. It is of interest to note that kynurenine was generally excreted by both normal and pyridoxine deficient dogs. Removal of the spleen from 3 dogs did not prevent the change in tryptophane metabolism following the development of pyridoxine

deficiency. It was also found that xanthurenic acid fed to either normal or deficient dogs was excreted unchanged.

The mechanism by which pyridoxine is able to stop short the oxidation of the metabolic product of tryptophane at the kynurenic acid stage is unknown and offers an interesting problem for future investigation. A search for other end products of metabolism which are oxidized more completely in the pyridoxine deficient than in the normal animal might yield comparable results of value in elucidating the mode of action of pyridoxine.

THE SKELETAL SYSTEM OF THE RAT DURING ACUTE STARVATION

I. Glickman, A. Morse, and L. Robinson (*J. Am. Dent. Assn.* **31,** *1435 (1944)*) have studied the effect of acute starvation upon the skeletal system of the albino rat. There was a generalized osteoporosis in the tibias, ribs, vertebrae, and jaws of the starved rats. The bone below the epiphysis of the ribs was made up of discontinuous, thin trabeculae, instead of the normal, parallel continuous arrangement. In the body of the vertebrae, the bone trabeculae were very small so that the marrow spaces were much larger than normal. Frequently the continuity of the trabeculae was broken and the trabecular margins appeared to be eroded and roughened. In the mandible, the vascular channels and marrow spaces were increased in width and volume. A definite loss of bone structure in the gingival crest of the interdental septa occurred which consisted of fragmentation of the bone matrix at the periphery with the formation of an irregular, sawtooth edge. There was little evidence of new bone formation in the starved rats to compensate for the generalized osteoporosis and bone destruction that was taking place.

When rachitic rats were starved, E. V. McCollum, N. Simmonds, P. G. Shipley, and E. A. Park (*Bull. Johns Hopkins Hosp.* **33,** *31 (1922)*) observed that calcium salts were deposited in the epiphyseal side of the metaphysis. This was equivalent to the reappearance of a provisional zone of calcification and the beginning of healing in the rachitic lesion. This process was identical with that observed when cod liver oil was given to rachitic rats. Similarly, J. P. Weinmann and I. Schour (*Am. J. Path.* **21,** *1047 (1945)*) observed a resumption of calcification in the dentin of rachitic rats which were starved for forty-eight hours or longer. The process of calcification appeared to be identical with that which occurred when either phosphate or vitamin D was given to the rachitic rats without starvation.

I. Glickman (*J. Dent. Research* **24,** *155 (1945)*) has described a series of experiments in which the effect of acute starvation on alveolar bone deposition was studied. In order to stimulate the development of alveolar bone, the upper left molar teeth of normal rats were removed. Then there was an extensive formation of elongated trabeculae along the alveolar bone margin immediately beneath the periodontal membrane of the lower left molars. Eruption of the lower left molars accompanied the formation of this new alveolar bone. No new trabeculae were observed on the unoperated side of the jaw. When the rats were acutely starved after the removal of the upper molars, a development of elongated trabeculae occurred on the alveolar bone supporting the lower molars on the side from which the upper molars had been extracted. Occlusal movement of the lower molars accompanied this new bone formation. These trabeculae were similar in distribution to those in the group of rats which had not been starved. However definite osteoporotic changes were observed in the alveolar bone immediately surrounding the newly formed trabeculae.

The results of McCollum, Simmonds,

Shipley, and Park, of Weinmann and Schour, and of Glickman present interesting examples of the ability of the rat to economize on its resources and to utilize them in local situations despite the presence of a systemic condition as drastic as acute starvation. During the acute starvation of rachitic rats, healing of the rachitic lesions of the long bones and calcification of the dentin could occur even though no vitamin D was given. The mineral salts for these processes must have been made available through the generalized osteoporosis of the skeletal system. Similarly when the upper molars of one side were removed, the local stimulus to produce new alveolar bone around the opposing molars on the same side was sufficiently great that it caused this bone deposition during starvation at the expense of the entire skeletal system. The full ability of the animal body to adapt itself to the many factors which make up its environment is unknown. The results described here are a slight indication of the great adaptation that occurs in as drastic a systemic disturbance as acute starvation.

LACTIC ACID BACTERIA IN NUTRITION RESEARCH

The tempo of progress in the isolation of any new vitamin depends less on its importance than on the methods available for its assay. The fact that concentrates of the antipernicious anemia factor must be assayed in man, for example, has so far proved an insurmountable handicap in attempts to isolate the active principle. Methods involving the use of small animals such as the rat or pigeon are unquestionably more practical but they are extremely laborious. The advent of microbiologic procedures has been a real boon for those interested in the isolation and purification of vitamins. Among the most useful microorganisms for this purpose and for the assay of foods, tissues, and other materials for known factors are the lactic acid bacteria.

E. E. Snell (*J. Bact.* **50,** *373 (1945)*) has discussed the use of these bacteria in vitamin research and has described investigations pertaining to their nutritional requirements. At first relatively crude sources of nitrogen were used. As successive essential nutrients were identified the pure substances or concentrates of them were added to the basal medium and a more highly purified source of nitrogen was substituted. As a result of these researches, there are now available, for most of these organisms, complete mediums in which all necessary constituents are pure, chemically defined materials. During the course of the work microbiologic assay procedures involving lactic acid bacteria proved to be of assistance in the isolation of pantothenic acid and of L. casei factor ("folic acid," "vitamin Bc") and similar assay methods are at present available for the quantitative determination of thiamine, riboflavin, niacin, biotin, pyridoxine, para-aminobenzoic acid, and some seventeen amino acids.

The history of this phase of nutrition began in 1936 when S. Orla-Jensen, N. C. Otte, and A. Snog-Kjaer (*Zentr. Bakt. Parasitenk. II*, **94,** *434, 460 (1936)*) showed that lactic acid bacteria require riboflavin and certain amino acids for growth. In the following year, E. E. Snell, F. M. Strong, and W. H. Peterson (*Biochem. J.* **31,** *1789 (1937)*) developed a new deficient medium by treating the peptone of an adequate medium with sodium hydroxide. This new medium, consisting of treated peptone, glucose, sodium acetate, cystine, riboflavin, and mineral salts failed to support growth of any of the test organisms unless small amounts of tissue extracts were added. The active principle in these extracts was later identified by E. E. Snell, F. M. Strong, and W. H. Peterson (*J. Am. Chem. Soc.* **60,** *2825 (1938)*) as pantothenic acid, which at that time was known

in concentrates only as a growth factor for certain strains of yeast. The effect of pantothenic acid on the growth of lactic acid bacteria was subsequently used extensively as a tool in the preparation on a large scale of concentrates of this vitamin, preliminary to its crystallization, identification, and synthesis. With a slightly modified medium, it then became possible to use L. casei for the quantitative determination both of pantothenic acid and of riboflavin.

For the study of additional nutritional requirements of lactic acid bacteria a concentrate of pantothenic acid was added to the medium and the sodium hydroxide treated peptone was replaced by an acid hydrolysate of purified casein, to which was added sufficient tryptophane to replace that destroyed during hydrolysis. When the new medium was inoculated with washed cells of L. casei or L. arabinosus only scanty growth occurred. The addition of niacin resulted in good growth which, however, failed on subculture in the same medium. E. E. Snell, and W. H. Peterson (*J. Bact.* **39,** *273 (1940)*) subsequently demonstrated that for successful growth of L. casei on this medium, three additional growth substances were required, two of which were identified as pyridoxine and biotin. The third substance was named, from certain of its properties, the "norit eluate factor." Lactic acid bacteria were used extensively as test organisms in the isolation of this factor which is now known by a variety of names including "the L. casei factor," "vitamin M," "folic acid," and "vitamin Bc."

It has been found possible to replace the casein hydrolysate of the medium with a mixture of purified amino acids. Seventeen of these have been shown to be essential for one organism or another of the group and one culture, Leuconostoc mesenteroides has been reported to require all seventeen (M. S. Dunn *et al., J. Biol. Chem.* **156,** *703 (1944)*). For many of the lactic acid bacteria, all compounds essential for growth are now known and may be supplied as pure crystalline materials.

The value of the lactic acid bacteria for the quantitative assay of various materials for B-vitamins and for amino acids continues. In addition it has been shown that substances as yet unidentified are stimulatory or essential for the growth of certain of these organisms and, in the words of Snell "It may be expected that further study of such substances will lead to additional findings of interest to both the biochemist and the bacteriologist."

A SURVEY OF HEMOGLOBIN LEVELS IN GREAT BRITAIN IN 1943

The soundness which has characterized the policies set by the authoritative nutritional advisers in Great Britain during the recent war has often been commented upon (*Nutrition Reviews* **3,** *249 (1945)*). An outstanding example of the thoroughness and the lack of biased thinking which should characterize nutritional surveys has now been provided by a report of the Medical Research Council's Committee on Haemoglobin Surveys (*Medical Research Council Special Report Ser. no.* **252,** *"Haemoglobin Levels in Great Britain in 1943 (with observations upon serum protein levels),"* *London, HMS Stationery Office (1945)*). This report is valuable not only for the actual data contained, but also because it illustrates the virtue of critical planning of such studies and the usefulness of an appreciation of certain fundamental statistical principles in the interpretation of the data obtained.

The Haldane-Gowers method of estimating hemoglobin (J. Haldane, *J. Physiol.* **26,** *497 (1901)*) was adopted because of its wide use in England and because of its simplicity. This method is based upon visual comparison by means of dilution

colorimetry of a standard and a solution of carboxyhemoglobin made from the unknown blood. Despite the fact that this method had been in general use in Great Britain for over forty years no systematic investigation had previously been made of the accuracy of the results or of the hemoglobin equivalent of the standard. In defining the "accuracy of the method" the two attributes considered were the deviation of the determined values from the "true values" and the deviation (or variation) of one determination from another.

All of the cooperating observers were supplied with apparatus previously calibrated by the National Physical Laboratory. The observers were tested by a series of bloods which were sent out from the National Physical Laboratory and the "true" hemoglobin concentration of which had been determined in that laboratory. It was found that the mean of the observer's results was 96.1 per cent of the National Physical Laboratory's "true" value and that 43 per cent of the results differed by more than 5 per cent from the National Physical Laboratory's ("true") results, 11 per cent by more than 10 per cent, and a small proportion by as much as 15 to 20 per cent. The personal variability ranged from a coefficient of variation of 1.02 per cent to 6.9 per cent. The various sources of error were analyzed and tabulated. The subjective source (color matching) appeared to introduce the largest single error, but the Committee felt that the use of a photoelectric colorimeter reduced this insufficiently to justify its employment.

The hemoglobin concentration measures by this method are expressed in "per cent." "One hundred per cent" is presumably equivalent to an oxygen capacity of 18.5 ml. O_2 per cent. This, in turn, is equal to 13.8 g. of hemoglobin (Haldane, *loc. cit.*). As the Committee notes, these factors have not been well defined.

The effects of venous constriction during the taking of blood on hemoglobin concentration and serum protein levels were studied by three independent groups. Evidence was produced which indicates that prolonged stasis tends to result in sampling blood with higher values of both hemoglobin and serum protein. The former differences are particularly marked when the initial hemoglobin levels are low. Constriction for half a minute resulted in no appreciable changes; hence, it is recommended that if a tourniquet is to be employed in securing venous blood it should be left in place not to exceed thirty seconds to prevent these phenomena.

The data which are reported were corrected for known errors and therefore are more comparable than data which have sometimes been secured in similar studies. In order to make comparisons with observations which had been reported by investigators prior to the introduction of wartime dietary restrictions the committee has applied "correction factors" for the apparatus employed by the various investigators where that apparatus could be obtained for checking. Inasmuch as such comparisons did not indicate a deterioration of hemoglobin levels during the war, this mass of comparable data on some 16,000 individuals is an important physiologic contribution.

The mean values for the larger groups of men (married or single) ranged from 101.7 to 103.3 per cent in the age range up to 50 years of age. A decrease in the mean hemoglobin values in men was noticeable in the 50 to 59 year age group. The "60 and over" sample had a mean hemoglobin concentration of 98.7 per cent.

The women, both married and single, had hemoglobin concentrations definitely lower than the men in all age groups and their distribution was more scattered as indicated by the greater standard deviation for women than for men. The mean values for single women ranged from 94.0 to 95.0 per cent in the single group before the age of 60. The means for the nonpregnant married

women ranged from 91.3 to 93.3 before 60 years of age. After 60 years of age the levels of both the single and the married women increased to 96.2 and 95.8 per cent respectively. It is of interest that the greatest variability occurred in the women in the age group 40 to 49 years. One interpretation of these findings which is offered is that during the childbearing period the lower hemoglobin level probably reflects the periodic (menstrual) blood loss and, hence, these figures may reflect a mild iron deficiency. This interpretation is strengthened by the rise in hemoglobin levels which is observed in the post-menopausal age group, the greater variation within the age group 40 to 49 years (during which time excessive menstrual bleeding is not uncommon), and finally by a study which indicated that in many cases the lower hemoglobin levels could be improved by appropriate iron therapy.

The hemoglobin data on the married women were analyzed according to age and number of pregnancies. The results can be summarized as follows: There occurred a progressive decrease in the mean hemoglobin values with an increase in the number of pregnancies; this change was evident in all age groups under 50 years; within a given "number of pregnancies" group the hemoglobin did not vary significantly with age; hence, age *per se* did not account for the lower levels. All groups showed an increase in hemoglobin concentration after the age of 50. Two possible suggestions are offered by the Committee to account for these observations. The first is that there is an unusually heavy demand for hemopoietic principles occasioned by numerous pregnancies. The other is that there is a decreased intake of hemopoietic substances by mothers of large families. The data do not permit a choice between these factors.

The studies of the 690 pregnant women demonstrated that it is not possible to arrive at a mean figure for all pregnant women. The hemoglobin concentration during pregnancy fell from a mean of about 91 per cent during the first months to an estimated 82 to 83 per cent during the seven to eight months. It was suggested that this fall is due to the increase in plasma volume which occurs during pregnancy. Further, it was noted that the mean hemoglobin level during the second pregnancy was slightly lower than during the first one.

The data on children indicate that the general trend is for a rise in the mean hemoglobin level from 77 to 78 per cent at the age of 1 year to a level of some 90 to 92 per cent at 6 or 7 years. At this level the mean plateaus until the ages of 12 or 13, where further increase occurs, to a maximum in the 16 to 19 year old boys. After puberty the girls have a value of 5 to 6 per cent lower than the boys.

These data, taken with the various other carefully planned studies of hemoglobin levels within apparently healthy populations (see *Nutrition Reviews* **3,** *202* (*1945*)), and coupled with data from extensive repetitive studies on the same individuals (for example, I. G. Macy, "Nutrition and Chemical Growth in Childhood," *Vol. II, Charles C. Thomas, Springfield, Ill.* (*1946*)) should lead to a better understanding of the variations to be expected in "normal" hemoglobin levels. If it is possible to extend these surveys to study the hemoglobin level of the population emerging from the shortages of war, as the Committee hopes, this valuable base line will be most useful in evaluating the influence of nutrition on near-maximum hemoglobin levels.

The Committee on Haemoglobin Surveys should be commended for a survey which might well serve as a pattern for similar investigations which may be carried out on an international basis by organizations such as UNO (see *Nutrition Reviews* **4,** *8* (*1946*)) in efforts to "collect, analyze, interpret, and disseminate information relating to nutrition, food, and agriculture."

THE VITAMIN D CONTENT OF HUMAN MILK

The effect of feeding massive doses of vitamin D on the content of this vitamin in human milk has been studied by L. J. Polskin, B. Kramer, and A. E. Sobel (*J. Nutrition* **30,** *451* (*1945*)). In a survey of 21 mothers who had received no vitamin D supplements during pregnancy, vitamin D could not be detected in the whole milk of 18 cases, while 17, 28, and 40 U.S.P. X1 units per liter were found in the milks of the 3 other mothers. Only slight increases were observed when massive vitamin D supplements were given during pregnancy. Thus when as much as 32,000 to 480,000 units were administered, no vitamin D could be detected in the milk of 7 out of 27 cases, and in 6 of the cases receiving the largest amounts, an average of only 53 units per liter of milk was found.

It appeared that somewhat more vitamin D occurred in the milk if the supplementation was given during labor or lactation. Thus in 2 cases where a single dose of 32,000 units was given to each during labor, levels of 91 and 216 U.S.P. X1 units per liter of milk were reached, and in 7 patients who were given daily doses of 40,000 units during labor and through the first seven to nine days of lactation, the vitamin D content of the milk ranged from 125 to 583 units per liter, with an average of 310 units.

Even the highest levels of vitamin D reached in the milk represent only a limited secretion ability. Thus in a balance study of a patient receiving 40,000 units of vitamin D daily during the first eight days of lactation, only about 1.3 per cent of the vitamin D administered was secreted in the milk. About 6.9 per cent was excreted in the stools, and 36.5 per cent was present in the blood fourteen hours after the daily supplement was given. The rest of the vitamin D was unaccounted for.

The same limited ability to transfer ingested vitamin D to the milk has been observed in the cow. H. Steenbock, E. B. Hart, F. Hanning, and G. C. Humphrey (*J. Biol. Chem.* **88,** *197* (*1930*)) supplemented one cow's diet with about 9700 I.U. of vitamin D per day as cod liver oil for a period of two years. The antirachitic property of the milk produced was not greater than that of the milk produced by the control cows. However, when a supplement of 50 g. of irradiated yeast (approximately 135,000 I.U. of vitamin D) was given to a cow daily, there was a definite increase in the antirachitic potency of the milk. When W. E. Krauss, R. M. Bethke, and W. Wilder (*J. Dairy Sci.* **16,** *549* (*1933*)) fed cows massive doses of vitamin D as cod liver oil, they also observed an increase in the vitamin D content of the milk. Similarly these same workers (*Ibid.* **17,** *685* (*1934*)) found that very large amounts of irradiated ergosterol or irradiated yeast had to be fed in order to increase the vitamin D content of the milk appreciably. For example, 120,000 Steenbock units of vitamin D as irradiated yeast had to be fed in order to obtain a level of 133 Steenbock units of vitamin D activity in the milk.

These data clearly indicate the limited ability to increase the vitamin D content of human and cow's milk. Even when the massive doses described by Polskin *et al.* were ingested daily by the mother, the antirachitic potency of the milk was insufficient to prevent completely rickets in the breast-fed infant. The amounts of vitamin D usually prescribed to meet the requirements of the mother and fetus during pregnancy and of the mother during lactation would have no appreciable effect on the vitamin D content of the milk. Even though there is such a limited ability to transfer vitamin D to the milk, the increased vitamin D requirement during reproduction makes a vitamin D supplement desirable if the mother and fetus are to maintain a normal calcium and phosphorous metabolism.

RELATION OF PROTEIN DEFICIENCY TO VIRULENCE OF AVIAN MALARIA

The relation of nutritional factors to infection has been demonstrated convincingly in malarial infection in birds. This subject as it is related to the role of various vitamins was reviewed previously (*Nutrition Reviews* **3,** *345* (*1945*)). In their latest report A. O. Seeler and W. H. Ott (*J. Infect. Dis.* **77,** *181* (*1945*)) describe studies bearing on the relation of protein deficiency to this type of parasitic infection.

One-day old, single comb White Leghorn chicks selected for uniform weight were fed one of four diets. Diet I supplied 1 per cent protein, Diet II 4.2 per cent, Diet III 7 per cent, and Diet IV 32 per cent. The diets were isocaloric, the amount of protein omitted in diets I, II, and III as compared with IV being replaced by an equal weight of dextrose. In other respects the diets were identical and contained all the nutrients required by chicks. Eleven-day old birds on each diet were divided into two groups of comparable weight, one to be infected and the other to serve as noninfected controls. Whole blood infected with Plasmodium lophurae was diluted with 0.85 per cent saline so that 1.0 ml. of the dilution would contain 50,000,000 parasitized erythrocytes. One-fifth ml. of this dilution per 50 g. of bird was given intravenously and the course of the infection was followed by parasite counts on blood smears made at twenty-four to forty-eight hour intervals.

The chicks receiving the three low protein diets gained no weight but survival was better the higher the protein content. It is of interest that the serum protein of the birds receiving the two diets poorest in protein averaged 1.6 and 1.7 g. per cent, respectively, whereas the average serum protein in those receiving 7 per cent protein remained at 3.5 g. per cent. This was essentially the same as the quantity found in the serum of the birds gaining weight at a normal rate and receiving 32 per cent protein in their diet.

Survival of infected birds was definitely correlated with the protein content of their diet. Of those that received the high protein diet none died during a twenty-eight day observation period. Of those receiving the deficient diets, survival was distinctly greater among the controls than in infected chicks. Furthermore, the course of the P. lophurae infection was strikingly different in the three groups on the protein deficient diet as compared with the birds receiving the high protein diet, for in the former the parasite count reached a much higher level and remained elevated longer than in the chicks receiving ample protein. Thus, the experiment showed that protein deficiency has a marked effect on the course of P. lophurae in the chick. Not only was the acute infection more severe in the protein deficient chick than in the chick on the diet of adequate protein content but the deficient bird was unable to clear the parasites from the blood stream as readily as the well nourished bird. P. lophurae malaria in chicks on a high protein diet was, in fact, a benign disease whereas the mortality was high in protein deficient birds. It is of interest that a protein deficiency, which was not sufficiently severe to decrease the total serum protein, still had a marked effect on the course of the avian disease.

It is of interest to speculate whether this susceptibility of the hypoproteinemic chick to malarial infection is related to an inability to form antibody corresponding to that which has been demonstrated in the hypoproteinemic mammal by P. R. Cannon, R. W. Wissler, R. L. Woolridge, and E. P. Benditt (*Ann. Surgery* **120,** *514* (*1944*)). If the effect of a protein deficient diet on human malaria is at all comparable to that observed in avian malaria, it offers at least a partial explanation for the severity and the persistence of malaria in the malnourished populations of the areas where malaria is endemic.

NOTES

Letter to the Editor

Dear Sir:

Through an oversight F. E. Volz, R. M. Forbes, W. L. Nelson, and J. K. Loosli (*J. Nutrition* **29,** *269* (*1945*)) failed to refer to an article by R. S. Harris, M. Clark, and E. E. Lockhart (*Arch. Biochem.* **4,** *243* (*1944*)). This same oversight has been carried into your summary of the "Effect of Soy Flour on the Biologic Value of White Bread Protein" (*Nutrition Reviews* **3,** *283* (*1945*)). The growth experiments of the rats Harris had here demonstrated that a white bread containing 2.3 per cent skim milk solids and 3.0 per cent full fat soya flour was superior to a bread containing 6 per cent milk solids not fat. The work of Volz *et al.* is a confirmation of this previous investigation.

Robert S. Harris,
Nutritional Biochemistry
Laboratories,
Massachusetts Institute of Technology,
Cambridge, Mass.

An Oxygen Analogue of Biotin

An analogue of biotin which contains oxygen in one ring in the place of sulfur has been synthesized by two laboratories (K. Hofmann, *J. Am. Chem. Soc.* **67,** *694* (*1945*); R. Duschinsky, L. A. Dolan, D. Flower, and S. H. Rubin, *Arch. Biochem.* **6,** *480* (*1945*)). K. Hofmann and co-workers (*Ibid.* **7,** *393* (*1945*)) suggest the name "oxybiotin" for this compound while Duschinsky *et al.* have termed it O-heterobiotin. There is no evidence at this time to indicate that this compound occurs naturally.

Reports from both of these laboratories indicate that this vitamer possesses less biologic activity than biotin. Thus, S. H. Rubin, D. Flower, F. Rosen, and L. Drekter (*Arch. Biochem.* **8,** *79* (*1945*)) found that their vitamer was 25 to 50 per cent as active for the growth of microorganisms as was biotin. The material showed some 5 per cent of the activity of biotin in the cure of egg white injury in rats. Similar results were reported by F. J. Pilgrim, A. E. Axelrod, T. Winnick, and K. Hofmann (*Science* **102,** *35* (*1945*)) and by Hofmann *et al.* (*Arch. Biochem.* **7,** *393* (*1945*)).

Rubin and co-workers (*loc. cit.*) found that this biotin analogue reacts with avidin and is inhibited by desthiobiotin, phenomena which indicate a fundamental relationship between biotin and the vitamer. These workers have also presented evidence which they interpret to indicate that the utilization of the analogue by yeasts depends upon its conversion to biotin—a mechanism which requires that the oxygen-containing ring of "O-heterobiotin" be opened and the oxygen replaced with sulfur. This concept raises the interesting question as to whether the sulfur containing ring in biotin ordinarily undergoes an opening and closing during metabolism.

Metabolic Balances

Methods for the accumulation, interpretation, and presentation of data pertaining to metabolic balances have received special attention and discussion by E. C. Reifenstein, F. Albright, and S. L. Wells (*J. Clin. Endocrinology* **5,** *367* (*1945*)). The report centers about metabolic balances of calcium, phosphorus, and nitrogen but it is valuable because of the consideration given to the general principles involved.

The importance of making only one change in the regimen at a time is stressed and it is pointed out that one must be alert for the introduction of unintentional changes. Thus, the influence of season on calcium balance is recalled and the means whereby errors so introduced can be pre-

vented are discussed. The basis on which the length of metabolic periods is chosen, the need for recognition of transitional periods between diets of differing content, and factors governing selection of a diet are considered. These investigators favor giving various minerals as medication when these are required in specific amounts, rather than recommending attempts to furnish the minerals by dietary means. It is pointed out that there may be marked differences between the analyzed content of diets and their content as calculated from tables in the literature.

Details concerning methods for collection of specimens and for preparation of specimens for analysis are presented. The interpretation of data receives considerable attention and there is a discussion of means whereby gross errors may be detected. What constitutes significant data and methods of presentation are discussed. Of special value is the discussion of various theoretical considerations and of the methods involved in computing certain derived data. These data include "theoretical" balances of phosphorus, calcium, nitrogen, potassium, and sulfur and "theoretical" weight curves.

Malnutrition in Occupied France

According to a summary of public health in occupied France from invasion to liberation by L. J. Besançon (*War Medicine* **8,** *283 (1945)*) the perfected technic of selective starvation employed by the Germans had resulted in hunger edema in insane asylums by the end of 1940. Since French people were actually, as well as traditionally, well fed, some of the effects of the reduced ration came as a surprise to physicians who had looked upon the classic deficiency diseases as tropical or exotic. Two situations mitigated against the worst results of the alarming deficit in food intake. One was the widespread contribution of the farms of northern and western France to the local dietary; the other was "family food parcels" received by certain persons with friends in the country. One of the chief shortages was animal protein.

Symptoms ranged from lassitude and weakness to hypersusceptibility to cold, chilblains, dyspepsia, achylia, atrophy of gastric mucosa, and pronounced diarrhea. Recovery was usually prompt when more food became available. In some instances, however, endocrine disorders in females resulted in precocious uterine involution. Growth in children was slow or actually stationary. Premature birth and high infant mortality were characteristic along with difficult breast feeding.

One of the peculiar signs was "an abnormally abundant diuresis in underfed patients," beginning with nocturia, but later becoming diurnal and reaching levels of 3.4 or 5.0 liters. It was not associated with undue thirst, and the urine was normal. It was not ameliorated with the occurrence of edema or hydrops. The widespread hunger edema was often preceded by weight loss, weakness, hypothermia, bradycardia, circulatory complaints, and polyuria. It was precipitated by muscular work, could be held in abeyance to some degree by bed rest, and was most frequent in men over 60 years of age. Treatment early enough with ample diet produced rapid relief but this was rarely possible and in the more prolonged cases an irreversible state developed where response could no longer be elicited. Though the heart, kidneys, liver, and blood vessels were not implicated there was a great increase in blood volume and extracellular fluids, with low total serum proteins. There was no response to thiamine.

Starvation osteopathy was first noticed because of bizarre spontaneous fractures, often bilateral and symmetrical, in such unusual sites as the middle portion of the ulna, the mandible, or the ribs. Sometimes generalized skeletal demineralization led to softening of the vertebrae with kyphosis and other changes which ran a rapid course from inception to marked deformity. Rickets once more became a problem.

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